Microbes, Immunity and Cancer in Capri – Another successful course of the EFIS-EJI Ruggero Ceppellini Advanced School of Immunology founded by Serafino Zappacosta

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Not many but much – '*Non multa sed multum*' – is the motto chosen in 1991 by Serafino Zappacosta and the other founders of the EFIS-EJI Advanced School of Immunology Ruggero Ceppellini. The motto was pertinent to the 29th Course of the School, where the participants enjoyed excellent lectures on the state-of-the-art of the meta-organism, that is the hybrid ecosystem made of our cells and microbes. Health and disease, including cancer, are properties of the meta-organism, and so are responses to new immunotherapies of cancer.

The Course was directed by Francesco Colucci (University of Cambridge, UK), Ennio Carbone (University of Magna Graecia, Catanzaro, Italy), Giorgio Trinchieri (National Institute of Health, USA), Laurence Zitvogel (Gustave Rouissy Cancer Campus, Paris, France) and Guido Kroemer (Centre de Recherche Des Cordeliers, Université Paris Descartes, Paris, France). The 25 attendants from 7 different countries and the 12 lecturers gathered at the Former Swedish Solar Observatory and now conference centre of the Italian National Research Centre (CNR) – *Osservatorio Cultura Ricerca Formazione Divulgazione* (**Figure 1, 2**), in the stunning surroundings of Anacapri, Capri Island, Italy – made available thanks to CNR members, Francesca Di Rosa (Institute of Molecular Biology and Pathology, CNR, Rome, Italy) and the President of the School Silvia Fontana Zappacosta (CNR, Naples, Italy). Nine of the participants received travel fellowships (**Figure 3**) made available by the European Federation of Immunological Society (EFIS), the European Academy of Tumor Immunology (EATI), and the Department of Experimental and Clinical Medicine of the

Magna Graecia University of Catanzaro, Italy. Fluidigm Corporation and Miltenyi Biotech sponsored the event. The introductory lecture was given by Ennio Carbone who talked about the historical background, the structure and the activities of the School (1).

Figure 1. The *Osservatorio Cultura Ricerca Formazione Divulgazione* of the Italian National Research Centre (CNR)

Figure 2. Introduction to the course: Laurence Zitvogel (left), Giorgio Trinchieri (Centre) and Guido Kroemer (right) introducing the course. On the background are the contact details of the EFIS Advanced School of Immunology Ruggero Ceppellini – founded by Serafino Zappacosta in 1991 – and its motto in Latin: "*Non multa sed multum*".

The theme of the 29th Course was "*Microbes, Immunity and Cancer*" and it was an idea of Giorgio Trinchieri, who in the 1970's trained with the great Ruggero Ceppellini himself in Turin and Basel. In his lecture on the origin of innate immunity as mediator of the crosstalk between microbes and host, **Giorgio Trinchieri** took the attendants on a fantastic journey from the primordial eukaryotic cells to modern innate immunity. Innate immunity evolved more than 2 billion years ago from the symbiosis of unicellular organisms with their microbes, which then became mitochondria. Diverse examples of symbiosis were offered, from unicellular organisms to deep-sea anglerfish (also known as sea-devil), to the Hawaiian bobtail squid and its glowing bacteria. All multicellular organisms live in symbiosis with microbes and in fact our bodies are meta-organisms harbouring about 100-fold more microbial DNA than human DNA. We respond to external stimuli, and so do our commensals, which, unlike us, can actually change their DNA through generations of offspring and under selective pressures, which adds to the complexity and flexibility of the meta-organism. Major roles have now been established for the microbiome in physiology, including in regulating metabolism, behaviour, and cognition, as well as in disease. Cancer, for example, is a disease of the meta-organism. Because innate immune cells do not

distinguish between commensals and pathogens, we ought to think that microbe-associated molecular patterns (MAMPs) – rather than pathogen-associated molecular patterns (PAMPs) – are the molecular mediators of the cross talk between microbes and host. Various experimental models were discussed, including the recent approach of using laboratory mice born to wild mice, which have natural microbiota and can model human immune responses (2).

Laurence Zitvogel gave a thought-provoking lecture titled "*The concept of Immunogenic Cell Death in oncology*". She pointed out that successful radio-, chemo- and even targeted-therapy may all in fact be immuno-therapies, because they induce immunogenic cell death (ICD). These therapies indeed stress cancer cells which, by dying of ICD, in turn, alert immune cells, particularly dendritic cells, which recognise stress through DAMPs (damage- or danger-associated molecular patterns) using their pattern recognition receptors (PRR). Indeed, distressed or altered self-antigens may be more important than neo-antigens on tumour cells to alert the immune system. The gut microbiota affects responses to chemotherapy and certain specific commensal bacteria provide adjuvants, and in so doing they turn immunological tolerance into immunogenicity (3).

Guido Kroemer gave an inspiring lecture on the importance of healthy nutrition, caloric restriction and autophagy for healthy ageing. For example, mice with deficiencies in the autophagy pathway become much more obese and even diabetic when put on high caloric intake though either fat diet or sugary drinks, with bad consequences on aging, cancer and cardiovascular diseases. Some micronutrients, for example spermidine delays aging in humans (4) and is produced by the microbiome and also found in some foods, including aged cheese, soybeans, peas, nuts, broccoli, mushrooms, pears and apples. Spermidine also reduces aging in yeast, nematode, flies, and mice. Kroemer explored the interactions between, on the one hand, responses to cancer therapies mediated by the immune system and, on the other, increased life span induced by autophagy, which can be triggered pharmacologically, genetically or through nutrition. The link between better responses to cancer therapies and improved life span is provided by 'caloric restriction mimetics' (CRM) and ICD inducers. Indeed, fasting, or CRM associated with ICD improves the outcome of cancer therapies in mice. His lab and collaborators are now applying a high throughput platform to identify new CRMs. The evidence that the microbiome influences immunotherapies of cancer was also reviewed. New research into mouse models of the genetically determined premature aging progeroid syndrome (Huntchinson Gilford) was shared, showing that progeroid mice lack the microbe *Akkermansia muciniphila*, which induces autophagy and is increased in healthy centenarians. In conclusion autophagy was put at the core of onco-metabolism, immuno-metabolism and whole-body metabolism,

including its microbiome, because autophagy 'dilates' time by increasing longevity, improving health and helping to treat cancer (5-8).

Junior faculty member **Oliver Kepp,** from the Kroemer lab, gave a detailed account of how cellular stress ignites anticancer responses and concentrated on the mechanisms and the clinical relevance of ICD. He also presented ways to couple a systems biology platform that screens compounds to ignite ICD with pre-clinical mouse models of immunotherapy using those compounds (9)

Junior Faculty member **Lisa Derosa** from the Zitvogel lab discussed how gut dysbiosis compromises the efficacy of immunotherapy in patients and in mice. She also discussed the emerging concept of oncomicrobiome and how this is a new opportunity in both research and to improve patient care. The challenge, which will require an extensive multi-disciplinary approach, is to identify a minimalist ecosystem that governs responses to the cancer immunotherapies.

Nicola Segata (University of Trento, Italy) gave an inspiring lecture titled "*Shotgun metagenomics for high-resolution investigations of the human microbiome*", where metagenomics is intended as the study of the total collection of uncultured microorganisms in an environment using high throughput sequencing. There are about 1M more microbes in and on us than there are people on our planet. It is clear, for example, that the gut microbiome is linked to several diseases, including liver cirrhosis, inflammatory bowel disease, obesity, type 2 diabetes and colorectal cancer. How the infant microbiome is acquired was also discussed. The gut microbiome is partially inherited at birth. There can also be vertical transmission of microbial genetic material from mother to child, but only for some sub-types of germs, as illustrated with *E. coli* phylotypes. Transmitted strains are also more stable. However, some of the microbiome is still unknown, for example about 40% of the skin microbial genetic material – dubbed "the dark matter" of our microbiome. Could this unknown microbial genetic material be the missing link between microbes and diseases? For what it can be seen in the known 60%, any two unrelated people share only about 35% of their microbiome, suggesting that the human microbiome is highly variable, unique and personal, and also largely uncharacterized. There may be thousands of unknown species and millions of unsampled genes. Elegant work was presented showing how to expand our horizon by large scale metagenomics across westernised and non-westernised populations, and by comparing modern and ancient humans (10, 11).

Petter Brodin (Karolinska Institute, Stockholm, Sweden) gave a fantastic lecture titled "*System-level analysis of immune-microbiome interactions early in life*". He made a strong

case for new technology now being available to enable us to study the human immune system as a system and over time, thus avoiding the reductionistic approach of studying one's favourite cell type at a given, fixed time. A critical time seems to be early in life, when the immune system develops also through exposure to the microbiome. Following the development of the immune system in newborns, his team has found a stabilisation of gut microbiome-immune system relationship at a specific time window early in life, which is reminiscent of the 'weaning reaction to the gut microbiome' in mice, and that may well be linked to the development of several conditions, including inflammatory bowel disease, asthma and various other allergies (12-14). Within the Human Protein Atlas Project, Petter Brodin also presented the new initiative "The Human Blood Atlas", which contains single cell type information on genome-wide RNA expression profiles of human protein-coding genes covering 18 cell types of B and T cells, NK cells, monocytes, granulocytes and dendritic cells isolated with cell sorting followed by RNA-seq analysis. In addition, the Human Blood Atlas also contains an analysis of the protein secreted and found in human blood and compared to various tissues.
(https://www.proteinatlas.org/humanproteome/blood)

Jolanda De Vries (Radboud University, Nijmegen, The Netherlands) gave an impressive lecture and presented unpublished results from the multiple clinical trials conducted in her centre using dendritic cell vaccines for patients with cancer (15).

Sebastian Kobold (Ludwig-Maximilians University, Munich, Germany) made a strong case for the need of progress in new cancer treatments, and in particular for T-cell therapies of solid tumours. He guided the attendants through the fascinating journey of the development of T-cell-based immunotherapies, culminating in the FDA-approved anti-CD19 CAR T cells immunotherapy for B-cell lymphoma in 2017. He also analysed the adverse effects and toxicity of CAR T cell-therapies and assessed the current state-of-the-art in treatments of solid tumours, including sarcomas, glioblastoma and pancreatic ductal adenocarcinoma. Despite many ongoing clinical trials, there are three major barriers to success: lack of T-cell entry into tumours, insufficient tumour cell recognition and local immunosuppression. New experimental approaches aimed at trespassing these barriers were discussed (16).

Junior Faculty member **Costanza Maria Cristiani** from Ennio Carbone's lab summarised new research on NK cells and cancer. She reviewed the evidence that NK cells recognize target cells in a complementary way to cytotoxic T cells and that they are particularly effective against cancer stem cells. In addition, NK cells can provide biomarkers to assist cancer immunotherapies, particularly for solid tumours (17).

Junior Faculty member **Amiran Dsutzev**, from the Trinchieri lab, discussed microbes and cancer. He provided evidence for how the genetic background can affect composition of gut microbiota and that it is mostly, but not exclusively, the innate immune system that impacts on the composition of the gut microbiota. For example, a deficiency in *Myd88*, *Il17* or *Ifng* predisposes mice to dysbiosis and development of colon cancer. The dysbiosis induced by *Myd88* deficiency in $CD11b^{+}$ cells is caused by loss of *Reg3g* production in colonic epithelial cells, resulting in *E.coli* outgrowth (18).

Junior Faculty member **Marie Vétizou**, also from the Trinchieri lab, discussed how dietary fibers affect responses to immune checkpoint blockers. She revised the evidence from both mouse and clinical studies that supports an important role for the gut microbiome composition in the modulation of responses to immune-checkpoint inhibition in cancer therapy. She also discussed recent progress like the importance of discovering microbiome-related biomarkers to predict patient responses across clinical centers. Moreover, fecal transfer from responder patients or healthy donors to non-responder patients could be implemented, if a favourable microbiome were to be identified. Other important issues are the characterization of both the mechanisms that make the microbiome enhance cancer therapy, and the perturbations that induce or maintain a favorable microbiome composition (19).

The informal atmosphere throughout the whole course facilitated discussions at the poster session, among the participants and the faculty (**Figure 4**).

Figure 3. The 9 recipients of the Travel Fellowships

Figure 4. The Faculty and the participant

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